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FOREWORD

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✓ For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

____In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

John W. Lowe, Executive Director Date

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INTRODUCTION

The objective of this grant is to establish, operate and manage research and teaching programs in overseas locations where the USUHS has established, or is in the process of establishing, bilateral research agreements. These centers are to serve as sites wherein research projects of programmatic interest to the USAMRDC in the field of tropical infectious diseases can be conducted by USAMRDC and USUHS personnel in collaboration with host national counterparts. The program also provides the opportunity to transfer technology to the host-country scientists and technicians through short-term and degree-granting programs. It also provides USUHS medical students, Master of Tropical Medicine and Hygiene students and Doctoral Candidates in Medical Parasitology and Vector Biology opportunities to obtain practical experience with tropical infectious diseases of the Western Hemisphere.

In Belize, the primary objective is to establish and maintain an Epidemiological Research Center (ERC) for infectious disease research and teaching in the Ministry of Health, Central Medical Laboratory (CML), Belize City.

Original research objectives were and are to:

1. Determine the etiology of acute febrile illnesses and jaundice; determine antibody prevalence against various arthropod-borne viruses (e.g., EEE, VEE, WEE, SLE, MAY, dengue, YF, VSV, etc.), leptospirosis, hepatitis A, B, C, D and E, by age, sex, ethnic group, and geographical location; maintain surveillance for epidemic disease due to arthropod viruses, especially dengue.
2. Determine prevalence of HIV and HTLV-1 infections in selected populations.
3. Determine patterns of drug resistance of *Neisseria gonorrhea* in various regions.
4. Determine patterns of malaria transmission; maintain surveillance for chloroquine-resistant *Plasmodium falciparum*.
5. Determine vectorial capacity of putative malaria vectors.
6. Address epidemiologic targets of opportunity (e.g., leishmaniasis in the Belize Defense Forces (BDF); causes of febrile illnesses in British Forces Belize (BFB), U.S. Army Corps of Engineers, etc.).
7. Determine the effectiveness of repellents and fabric impregnants for protection of deployed troops from endemic vector-borne diseases.

8. Validate the remote sensing models developed for use in predicting temporal and spatial changes in malaria vector abundance in Mexico, in a second ecologically similar area.

Background

Belize is located on the eastern coast of Central America at the base of the Yucatan Peninsula, surrounded on the west and north by Guatemala and Mexico and on the east by the Caribbean Sea. The jungle covered Maya Mountains occupy the southwestern portion of the country, rising to 1122 feet at Victoria Peak; the remainder of the country is low, crop or scrub-covered coastal plains. Belize was founded as a buccaneer settlement and entrepot. Today, Belize is an English-speaking country, having gained its independence from Great Britain in 1981. The population is estimated at approximately 175,000 people, and is made up of a mixture of Mayans, Garifuna (Afro-Amerindian), Blacks, East Indians, Creole and Caucasians. About half of the population resides in the major City, Belize City, located on the Caribbean coast. The capital, Belmopan (population approximately 5000) is inland and was built as a Federal District after a devastating hurricane in 1961 destroyed the previous capital, Belize City. There are several other smaller cities; i.e., Punta Gorda, Stann Creek, Hill Bank, Orange Walk, San Ignacio and Indian Church, scattered through the coastal plain.

Medical care is provided by a socialized medical system and is centered around local health clinics and district hospitals in the smaller cities and towns and a large central hospital in Belize City. Emergency cases (largely surgical) are brought to the Belize City Hospital for care. The Belize City Hospital was built about 1930 and is a two-story building backed on the sea. It has about 200 beds, of which about 60% are dedicated to acute surgical patients. The hospital is divided into male, female and pediatric wards for both surgery and medicine. There are, in addition, neonatal and intensive care wards. A small chemistry, hematology and immunochemistry laboratory used for acute diagnostic procedures is located in the hospital. The majority of diagnostic and public health laboratory procedures are performed at the Ministry of Health, Central Medical Laboratory (CML) located about 3 miles north of the city. Current laboratory capabilities include: malaria smears (approximately 29,000 per year), bacteriologic cultures, routine biochemistries, and HIV antibody testing using commercially available ELISA kits.

Febrile Illnesses

Little is known about infectious diseases specific to Belize itself. Based upon limited information from Belize and other Central American countries, it may be inferred that in Belize, tropical infectious diseases are common. Yellow fever has been known to occur in the Yucatan¹, dengue and malaria are endemic in Belize², and cutaneous leishmaniasis, in almost epidemic numbers, has been reported in British troops stationed in Belize³. Cutaneous and visceral leishmaniasis have been reported in nearby Honduras and Guatemala⁴. Leptospirosis, and Venezuelan equine and St. Louis encephalitis have also been found in neighboring countries⁵. Enterically transmitted non-A, non-B hepatitis has recently been identified in Mexico. The Statistics Department at the hospital reported yearly admissions over the past several years for enteric fever of 4 to 6 cases; jaundice, 60 cases; and fever of unknown cause, about 150 cases. A great deal needs to be done to determine the prevalence and the incidence of tropical infectious diseases agents in Belize.

Cases of unexplained fever were selected by trained Belizean collaborators from the patients over 12 years of age presenting at the Belize City, San Ignacio and Orange Walk Hospitals. Patients with sickle cell disease, meningitis, dysentery, or evidence of peritonitis, wound infection, pneumonia, tuberculosis or HIV infection were not included in the study. Thick and thin malaria films were prepared from a finger stick and examined. Patients positive for malaria were listed but not studied. Patients selected for the study were divided into two groups, those with and those without jaundice. A systematic clinical exam was performed and blood was obtained for diagnostic tests. Sera were analyzed for the presence of antibodies to arthropod-borne viruses, leptospirosis and hepatitis. Several new patients were added to this protocol in 1992, but serologic results are still pending.

Leishmaniasis

Cutaneous leishmaniasis is a zoonotic disease transmitted to man by human-biting female phlebotomine sand flies. Many *Leishmania* strains belonging to at least four species are capable of causing human disease. The clinical manifestations of the infection are primarily species-dependent, but a number of poorly defined host factors may influence disease expression. Flagellated promastigote-stage leishmanial parasites develop in the gut of female sand flies and are transmitted to the

vertebrate host during a blood meal. Promastigotes rapidly parasitize macrophages, convert to the intracellular amastigote stage, and multiply. In the absence of specific immunity, they circulate to the regional or systemic reticuloendothelial system and ultimately cause sores and other manifestations after an incubation period of 1-6 weeks or longer. In the New World, the main species are *L. mexicana* and *L. braziliensis*. Over a dozen different strains and subspecies have been found to be capable of causing human disease.

In the New World particularly, cutaneous leishmaniasis presents with skin lesions as a major manifestation, but regional lymphatic chains are frequently involved as well. Typically, erythematous macules appear at the inoculation sites up to months after infection, followed by papules. The papules subsequently become nodular and may ulcerate to form well circumscribed ulcers with indurated margins and necrotic eschar-covered bases. In the absence of bacterial superinfection, which is unusual, the lesions are not painful. Satellite lesions may be found a small distance from the primary ulcers in otherwise normal-appearing skin. Some strains appear to cause a milder form of the disease, and the primary lesions may not ulcerate but instead present as nodules, papules or eczematous plaques. Nontender nodules and inflammation can develop along draining lymphatics. Regional lymphadenopathy is common, and parasites can be isolated from these nodes. Metastatic spread in the New World is generally associated with *L. braziliensis* and may occur months or years after acquisition in up to 80% of infections. Infections caused by *L. mexicana*, *L. peruviana*, and some strains of *L. braziliensis* are thought to cause localized disease without mucous membrane involvement. Spontaneous healing appears to be quite common with *L. tropica*, *L. major* and *L. mexicana*, except when the ear (pinna) is involved.

Leishmania mexicana is primarily distributed in Mexico, Belize and Guatemala. It is commonly (in 40% of cases) associated with lesions limited to the pinna of the ear and classically occurs in those harvesting gum from chicle plants, hence the name, "chiclero's ulcer." Forest rodents are the natural reservoir for *L. mexicana*. *Leishmania braziliensis* is thought to occur in Guatemala, though its presence is not as well documented as that of *L. mexicana*. In the Old World, cutaneous leishmaniasis is typically ulcerative and in many cases heals spontaneously within several months. Spontaneous healing of New World lesions is much less predictable. Certain South and Central American species, notably *L. braziliensis*, may result in a slowly healing primary ulcer and the late development of a mutilating infection of the upper respiratory tract (mucocutaneous leishmaniasis or espundia). A rare form of leishmaniasis known as diffuse cutaneous leishmaniasis is characterized by massively parasitized nonulcerated nodules, specific cutaneous anergy, and a poor

response to therapy. It occurs in both the New and Old Worlds. Serious illness with leishmaniasis has been reported as a manifestation of HIV infection.

The differential diagnosis of cutaneous leishmaniasis includes pyogenic bacterial, mycobacterial (*Mycobacterium marinum*), fungal (blastomycosis, sporotrichosis, histoplasmosis) and spirochetal (yaws and syphilis) infections, plus lupus erythematosus, sarcoid and malignancy.

Following the outbreak within the Belizean Defence Force in 1990, cases have been closely monitored.

Gastroenteritis

Rotavirus is the major cause of severe diarrhea in children. Worldwide it is estimated that there are 140 million cases, with 1 million deaths⁶. Approximately 50% of infants and young children hospitalized with diarrhea are infected with rotavirus⁷. Transmission is thought to be by the fecal-oral route. Seasonality is a poorly understood feature of rotaviral enteritis. In temperate climates, rotavirus is detected most frequently in the winter; however, in the tropics it is detectable all year around with seasonal trends that are less distinct⁸. LeBaron, *et al.*⁶ and Glass⁹ predicted that the rotavirus peaks (2 highest consecutive months) for Mexico, Belize and Guatemala would occur in the October - November time frame.

Hepatitis

In May 1991, concern that cholera may be spreading north through Central America prompted officials of the Belizian Ministry of Health to initiate an active search for possible cases. In the Cowpen area of southeast Belize, there were no reports of illness resembling cholera, but banana farm workers and local health officials were concerned about the large number of people who were or had been ill with hepatitis. In some cases people had diagnosed themselves as having hepatitis, based upon the occurrence of jaundice. For others, the diagnosis had been made by local health professionals.

Cowpen residents considered the appearance of hepatitis to be an annual occurrence, associated with the dry season, and attributed the disease to the poor quality of the water available. However, many believed the 1991 dry season to be more severe and prolonged than usual and the numbers of cases, particularly in young adults, to be greater than that observed in past years. Concern about hepatitis heightened when the

death of a young, pregnant woman and her child were attributed to the disease.

The Belizian Ministry of Health responded to the apparent increase in hepatitis cases by requesting assistance from the Belize - United States Epidemiologic Research Center in Belize City. In collaboration with the Uniformed Services University of the Health Sciences and with laboratory support from the Walter Reed Army Institute of Research, a preliminary investigation was done in May 1991 in an attempt to establish a diagnosis. The May investigation by the Belizian team established the presence of hepatitis in the Cowpen area and the need for further study. This was followed in June 1991 by an expanded effort to identify and define cases and to do a cross-sectional study of hepatitis markers and related variables in banana farm workers and their families.

Larval Ecology

As with most Central American countries, the numbers of malaria cases in Belize has increased dramatically in the last few years. Belize presently reports the highest annual parasite index of any Central American country¹⁰.

The vectorial roles of the different anopheline species present in Belize are poorly defined, even though several endemic species are known vectors in other geographical areas. At present, *Anopheles albimanus* is considered to be the primary malaria vector in Belize. Although there is evidence that this species is indeed a primary vector, the roles of other species that occur on the coastal plain, and in the foothills and mountainous areas should be delineated. Improved understandings of the biologies of the different vectors in different geographical areas should lead to improved targeting of malaria control interventions.

Mosquito surveys and characterization of their aquatic habitats were conducted in Belize during wet and dry seasons of 1990 through 1992. The primary objective was to collect taxonomic series of mosquito species found in Belize, with secondary emphasis on determining the presence/absence and quantifying the abundance and phyoeological relationships of anophelines in various aquatic habitats.

Tick-borne fever

The archaeologists, Drs. Elon and Dianne Chase, at Caracol, a Mayan Ruin in Mountain Pine Ridge, expressed concern over the number of tick bites being reported by the students and workers (one man, "tick man", had 134 ticks removed one day). Sera were collected from 47 individuals for analysis. Their concern was that febrile illnesses could be due to Lyme

disease, heretofore unreported in Belize, and also about the possible cause of a viral-like syndrome experienced by workers last spring.

Cholera

The first case of cholera in Belize was confirmed on 10 January 1992, in Punta Gorda. By 28 January 1992, two more cases were confirmed from Punta Gorda. Colonel Krieg accompanied an epidemiological team investigating these cases. The team included epidemiologists from Guatemala, Jamaica and Belize. All three of these first cases seemed to have been imported, probably from Guatemala.

More cases of cholera continued to be reported from Toledo District and later, from Cayo District. By 7 September 1992, there were a total of 29 reported cases (including two deaths). As of 26 November 1992, there were a total of 189 reported cases (including three deaths). These cases were mainly from southwestern Toledo District, but a few cases have been reported from several towns in Cayo District (mainly along the Western Highway) as far east as Belmopan.

At the request of the Permanent Secretary for the Ministry of Health, Mr. Fred Smith, the ERC developed a detailed plan and a "costed" list of supplies and equipment needed to train District medical technologists in the proper identification of cholera organisms in medical samples. These were offered to the CML on 13 October 1993, along with the services of two ERC technologists to do the training. A limited amount of such training has been started by CML personnel.

RESULTS AND PROGRESS

Administrative

Colonel Krieg presented a microbiology course at the Ministry of Health (MOH) Nursing School. He briefed four U.S. Air National Guard units and several consultants on missions of the MOH and the Epidemiologic Research Center (ERC). He attended meetings of the National Epidemic Prevention and Control Committee, the MASICA (Medio Ambiente y Salud Para El Istmo Centro Americano) Executive Committee and weekly U.S. Embassy Country Team meetings.

Lieutenant Commander Rich Haberburger and Carl Harding, Naval Medical Research Institute, Bethesda, MD, initiated the protocol entitled, "Epidemiological Survey of Acute Diarrheal Disease Among Belizean Children," in January 1992.

Dr. John Cross and J. Fred Duncan, both from PMB, USUHS, Bethesda, MD, presented five workshops on malaria to medical personnel in Belize. These were requested by the MOH and were funded by the Pan American Health Organization (PAHO). They focused on the collection and processing of blood for malaria diagnosis, and the identification of malaria species in such samples.

Three Belizean physicians returned to Belize from the USUHS to complete their MPH/MTM&H degree field projects. Captain Peter Craig, M.D., Medical Officer for the Belize Defence Force (BDF), and G. Mohan Kishore, M.D., Administrative Officer for the Director of Health Services, MOH, completed their Master of Tropical Medicine and Hygiene (MTM&H) degrees. Dr. Michael Pitts, General Medical Officer, Matron Roberts Health Clinic, completed his Master of Public Health (MPH) degree.

Two MS-IV students completed rotations in tropical medicine in December 1991 and June 1992 at the ERC and MOH facilities. Each worked at the ERC, Belize City Hospital, and MOH clinics, and conducted a walk-in clinic in Cowpen, where donated food and medications were also distributed. They also helped investigate an outbreak of hepatitis in Valley of Peace, a refugee settlement in Cayo District [about 5,000 people; mainly families (many of them Mayans) from El Salvador, Guatemala, and Honduras], and helped present malaria workshops in the districts.

Posters on ERC activities and gastroenteritis, and a video tape from the 1991 "teleconference" (between USUHS and clinicians in Belize), were presented at the annual meeting of the American Society of Tropical Medicine and Hygiene in Boston, MA, 1 - 5 December 1991. These were also displayed at the National

Agriculture Show, Belmopan, Belize, 1 - 3 May 1992, and during Health Week at the Nursing School, Belize City, Belize. Starting in January, the ERC staff provided more than 40 weekly continuing medical education (CME) classes on a variety of clinical laboratory subjects to interested medical personnel throughout calendar year 1992. Two ERC technologists, Ms. Linda Reyes and Ms. Shilpa Hakre, attended the two-week Continuing Education Diagnostic Parasitology Course at USUHS, Bethesda, MD, July 1992.

Members of the ERC staff attended Belize's National Agricultural Show, 1 - 3 May 1992; the 37th Annual Commonwealth Caribbean Medical Research Council (CCMRC) Meeting, 22 - 25 April 1992, Curacao, Netherlands Antilles; and the Society of Armed Forces Medical Laboratory Scientists (SAFMLS), San Antonio, TX, 12 - 16 April 1992. They also attended a PAHO-sponsored HIV workshop and the Belize Medical and Dental Association's 11th Annual Congress, 1 - 3 October 1992, both in Belize City.

A USUHS employee on TDY to present a malaria workshop had a myocardial infarction during a trip to Dangriga. The assistance of the British Forces Belize (BFB) was instrumental in stabilizing and evacuating him to Wilford Hall USAF Medical Center, San Antonio, TX. A Royal Air Force (RAF) flight surgeon was flown in a RAF helicopter to Dangriga to transport the patient to the BFB hospital. They provided constant support throughout his stay and during his transport to Belize International Airport for evacuation. The Hospital Commander was outstanding in assisting with the coordination for the evacuation, and she accompanied the patient to San Antonio. The 18 hours of work was truly a team effort involving ERC staff, Belizean friends, U.S. Air Force (especially Howard AFB, Scott AFB and Lackland AFB) and the BFB.

MAJ Robert E. Miller was the ERC's Acting Co-Director, 10 July - 7 September 1992, enroute (PCS) to WRAIR. LTC Harold J. Harlan was ERC's Acting Co-Director, 7 September - 12 December 1992. Maj Miller coordinated with Dr. Errol Vanzie, Director of Health Services (DHS) and Co-Director, ERC, to help establish an Institutional Review Board (IRB) for human use studies in Belize. Dr. Lopez, Chief Epidemiologist, is the MOH's permanent Chairman of this IRB. LTC Harlan provided Dr. Vanzie a current copy of the Memorandum of Understanding between the Governments of Belize and the United States for review. LTC Harlan gave Dr. Mohan Keeshore, Assistant, DHS, three protocols [revised hepatitis surveillance, LTC Ken Hoffman, M.D.; screening health care workers in Belize City Hospital for HBV, Linda Reyes, B.S. and Shilpa Hakre, B.S.; and screening of donor units at the Belize City Hospital for

Chagas' disease, Ruth Jaramillo, B.S., M.T. (ASCP)] for coordination and review by the IRB.

In September 1992, CDR Joe Bryan, M.D., USUHS, gave a presentation on human hepatitis infections and CPT Peter Craig, M.D., presented results of his study of hepatitis marker prevalence in the BDF, to Belize City Hospital and MOH medical professionals.

The ERC conducted surveillance for Asian Tiger Mosquitoes, *Aedes albopictus* (Skuse), at 10 sites where it is likely to have been introduced into Belize [Belize City port, the international airport, and BDF sites at Price Barracks, the Maritime Wing (Belize City) and Camp Belazario (San Ignacio)]. Ovitraps were monitored weekly, 19 October - 4 December. No Asian Tiger Mosquitoes were found. Collections included *Aedes aegypti* (6 sites), *Culex quinquefasciatus* (8 sites), and *Wyomyia* (1 site), with a few specimens yet to be identified.

LTC Harlan collected, reared and tested larvae of four vector/pest mosquito species for susceptibility to commonly used insecticides. He has also tested adults of two of these same species. All were tested using standardized WHO "diagnostic dose" procedures and previously published or suggested dose levels. Technical equipment, chemicals, rearing supplies and technical guidance were all provided by USAEHA, Aberdeen Proving Ground, MD, or USAEHA-N, Fort Meade, MD. Data compilation and analysis are currently still in progress, but initial results show that tested populations of *Ae. aegypti*, *Aedes taeniorhynchus*, *Anopheles albimanus*, and *Cx. quinquefasciatus* larvae were all susceptible to temephos, malathion, dursban, and cyfluthrin. Adult *Cx. quinquefasciatus* and *Ae. taeniorhynchus* were susceptible to DDT and malathion.

LTC Harlan ran gravid adult mosquito traps for seven trap-nights at sites in Belize City. Specimens will be kept on dry ice for later shipment to CDC, Fort Collins laboratories for virus isolation attempts and DNA-probe species identification trials.

LTC Harlan and Ms. Reyes presented a 1-hour laboratory demonstration of indirect fluorescent antibody (IFA) detection of pathogens and use of an ultraviolet microscope to Dr. Hall's microbiology class at the University College of Belize, 18 September 1992. Three ERC medical technologists presented a 2-hour talk on blood banking procedures and techniques to a third-year biology class of Pallotti High School, Belize City, 17 November 1992.

The ERC, with much help from key people at USAEHA-N, USUHS, and WRAMC, provided the BDF (Dr. Craig) with specific ordering and price information for vector/pest control equipment and chemicals,

and for U.S. military standard eyeglass frames and accessories. Five practical technical references (with keys), handbooks, and several small technical items were also procured for future ERC use.

Febrile Illness

Demographic data and sera were collected on 22 patients at the Belize City Hospital. Serologic examinations were accomplished at USAMRIID, where the procedure is to examine sera for IgG, and where results are positive, to examine acute phase sera, if available, for IgM to the same antigens. Results on acute phase sera for IgM are still pending. Results of examinations of single sera for IgG are shown in Table 1.

Table 1. Prevalence of viral IgG antibodies in patients with FUO at the Belize City Hospital in 1992.

<u>Antibody</u>	<u>No. of Patients</u>
EEE	3
VEE	4
WEE	0
MAY	2
DEN2	4
DEN4	0
SLE	6
YF	0
VSVI	0
VSVNJ	0
TOTAL REACTORS	19
TOTAL PATIENTS	22

From 21 February - 15 May 1992, 160 soldiers from Company A, 52d Combat Engineer Battalion (Heavy), Fort Carson, constructed a bridge over Soldier Creek, Belize. Blood samples were collected before deployment, and again, before return to the U.S. There were no significant illnesses in the group; however, there were soldiers that converted to being antibody positive during their deployment (Table 2).

Table 2. Soldiers that converted from negative to positive for presence of IgG arboviral antibodies.

<u>Antibody</u>	<u>Number of Soldiers</u>
EEE	4
VEE	1
WEE	1
DEN2	1
SLE	19
CHG	7

Leishmaniasis

The incidence of leishmaniasis in the Belizean Defence Force has dropped significantly, with no cases reported in the past 12 months. This is apparently a reflection of effective preventive education and the use of repellent. One suspected case was later ruled out by the treating physician. The soldier who did not respond to treatment with the paramomycin ointment and who was medically evacuated to Walter Reed Army Medical Center for treatment with IV Pentostam, has recovered.

Gastroenteritis

The 1991 outbreak of gastroenteritis developed over a wide area of Belize¹¹. Although, the team did not travel to the southern districts, the District Medical Officers indicated that there had been no discernible increase in the number of cases of diarrhea and vomiting. The incidence peaked during the last week of February and the first week of March. Over 69% of the reported cases in the outbreak were ≤ 4 years of age, and there was no significant difference in sex. The age distribution was compatible with a rotavirus etiology.

It had been reported that the yearly outbreak of gastroenteritis in this area of the Americas should appear in the late fall, October - November⁶. In order to determine whether this outbreak represented an unseasonal outbreak or a seasonal outbreak at a time different from other countries studied in this region, the clinical records for the Matron Roberts Health Clinic (MRHC), Belize City, were reviewed back to January 1989. The cases of gastroenteritis reported were stratified by month, age and sex. The number of detections for the

month was compared to the mean number of detections (53.6 cases/month) over the 28 months of data collected. The peak activity occurred January - May 1989; February March, May, July and August 1990 and January - March 1991. For September - December 1988, 15, 22, 31 and 35 cases were reported, respectively, well below the mean number of detections/month. The two consecutive months in each year with the highest incidences were January - February 1989, February - March 1990 and February - March 1991. Approximately 45% of the cases were in infants and children ≤ 4 years of age with no significant difference by sex.

The outbreak would appear to be seasonal, with an increase in incidence in January - May and a peak incidence during January - March. This coincides with the rainfall pattern in Belize. The dry season in Belize begins in January (144.8 mm) and continues through February (68.6 mm), March (40.6 mm), April (61.0 mm) and May (127.0 mm). These figures are a 10-year average for Belize City. There is also a climatic anomaly (the "little dry" or "mauger" season) in August when the rainfall is below average. This coincides with the increased incidence of gastroenteritis seen in July and August 1990. The apparent correlation with the dry season has been reported for enteric fevers in developing countries¹². This is apparently a result of the drying up of drinking water sources with a concomitant concentration of waterborne organisms. These primary water sources may in themselves become the source of infection, or contaminated, secondary water sources may be responsible. In the case of many of the villages in the area of the outbreak in Belize, as the vats dry up, water is obtained from streams or rivers, which are often contaminated.

Rotavirus appeared to be responsible for a significant number of cases in the outbreak of gastroenteritis (Table 3), although there undoubtedly were other causes. In addition to a *Shigella flexneri* isolate, there were a number of cases that were negative for rotavirus, and no bacterial or parasitic pathogen was identified. It is possible that the stool specimens were obtained when the concentration of rotavirus was below the sensitivity of the ELISA kit or that some other undetermined cause was responsible. In most cases of rotavirus infection, the viruses are not detectable after the eighth day of illness¹³. Some of the stool specimens were obtained 7 or more days after onset of illness.

The hospital and clinic-based, case-control study of acute diarrheal diseases in children was initiated in January 1992. During the two weeks the investigators were in Belize, the incidence of

diarrhea was much lower than expected based on data analyzed during the outbreak of diarrhea caused by rotavirus in 1991. Specimens were obtained from 36 patients and 17 controls. Rotavirus was the most frequently detected pathogen among patients (43%); however, a higher percentage of controls tested positive (57%). A higher percentage of controls also was positive for *Giardia lamblia* (43% versus 14%). There were 5 (14%) cases each of *Salmonella spp.*, *Vibrio spp.* and *Plesiomonas spp.* infections in patients, and there were no bacterial pathogens detected in the controls. Stool specimens continued to be collected for one month after the two-week study period. Samples from 133 patients were analyzed for enteric pathogens. Ova and parasite, rotavirus and adenovirus screens were not done on many of the specimens because patients would/could not provide fecal specimens and only anal swabs were obtained. Once used for bacterial cultures, the swabs are unsuitable for O&P and viral screens. There was one *Salmonella spp.* and three *Shigella* infections. Twenty-nine (53%) of the 55 samples tested for rotavirus were positive. None of the samples tested were positive for adenovirus. Four fecal specimens were positive for *Giardia*. The antibiotic sensitivity testing of bacterial pathogens and typing of *Escherichia coli* isolates are still in progress.

The preliminary analysis indicates that children in Belize may be healthy carriers of both rotavirus and *Giardia* and probably contribute to the perpetuation of diarrheal illnesses due to these agents.

Table 3. Results of bacterial, ova & parasite, and rotaviral analysis of stool specimens from 1991 outbreak of gastroenteritis.

<u>Location</u>	<u>Number of Patients</u>	<u>Rotavirus Positive</u>	<u>%</u>
Blue Creek	2	1	50
Belize City	14	10	71
San Pedro ¹	12	5	42
Succotz	8	1	13
Valley of Peace ²	9	6	67
TOTAL	45	23	51

1 One patient was positive for *Shigella flexneri*.

2 Two of the patients positive for rotavirus were also positive for O&P (*Ascaris* in one and *Ascaris* and *Trichuris* in the other).

Hepatitis

COWPEN STUDY

In this study, we found a high prevalence of people with prior exposure to HAV and HBV, with new cases of HBV occurring. Additionally, we found a large proportion of people who are potentially infective for HBV. We could not establish a serologic diagnosis in most of our collected cases of hepatitis. These cases can be characterized as non-A, non-B, non-C, non-D hepatitis, and could be hepatitis E cases; however, ELISA tests of sera for HEV in Dr. Purcell's laboratory and by Genelabs have yielded only inconsistently, weakly positive reactions in a few sera.

Hepatitis A and E have a fecal-oral route of transmission. Prevention is strongly dependent upon a purified water supply system, good personal hygiene practices and proper disposal of human waste. Our study would indicate that hepatitis A seems to be an illness of the very young in Cowpen; HEV could conceivably be a newly introduced virus attacking the young adult population.

Defining an attack rate for HEV is complicated by the fact that the testing for E is still in the developmental research phase¹⁴. The incidence of E may be high but not detectable using current technology. Alternatively, we may be dealing with a previously undescribed virus.

Our study reflects hepatitis B exposure has occurred in 73% of the Cowpen population, and 16% are positive for HBsAg. Clinical jaundice was most often due to new hepatitis B infection. There was no evidence that delta particle was present in any clinical case. A small number of people in our population showed evidence of exposure to HCV. Infection by hepatitis C appears to occur independently from B and is a frequent complication of transfusions¹⁵. The initial report was presented at the 37th Annual Commonwealth Caribbean Medical Research Council Meeting, 22 - 25 April 1992, Curacao, Netherland Antilles, and has been accepted for publication¹⁶.

The ERC attempted follow-up serum sampling of 56 persons in the Cowpen area whose initial samples (18 months earlier during a hepatitis study) had been inconclusive. Only 18 could be determined to still live in the Cowpen area; 13 were sampled, 2 refused, 3 could not be located despite over 32 man-hours of effort. The serology results are pending.

HEPATITIS SCREENING FOR THE BELIZE CITY HOSPITAL BLOOD BANK

A total of 1,337 samples were submitted to the ERC by the central blood bank during 1992, for determination of anti-HAV, HBsAg and anti-HBc. All tests were performed by the ERC staff using ELISA. The results are shown in Table 4.

Table 4. Results of screening blood bank donor units for hepatitis markers.

<u>Test</u>	<u>Number Tested</u>	<u>Number Positive</u>	<u>% Positive</u>
HBsAg	1,243	67	5.4
anti-HBc	282	71	25
anti-HAV	709	662	93.4

HEPATITIS MARKER PREVALENCE IN THE BDF

Peter Craig, M.D., with the assistance of Joe Bryan, M.D., USUHS, conducted a study of the prevalence of hepatitis markers in blood sera of active duty BDF personnel¹⁷. This was his MTM&H research project. The ERC staff helped Dr. Craig collect 503 blood samples from active duty BDF soldiers at Price Barracks, Ladyville; the Maritime Wing, Belize City; and Camp Belazario, San Ignacio. Table 5 is a summary of the results.

Table 5. Results of screening 503 BDF soldiers for hepatitis markers.

<u>Test</u>	<u>Number Positive</u>	<u>% Positive</u>
HBsAg	19	3.7
anti-HBc	147	29.2
anti-HAV	456	90.7

The prevalence rates of antibodies to hepatitis A did not vary significantly by aGe, rank and ethnicity. Rates of anti-HBc varied significantly among the ethnic groups, with the lowest rates in Mayan Indians (5%) and Mestizo (7%), and significantly higher rates among Creole (30%) and Garifuna (54%). Rates increased with increasing age from 25% in those 18-24 years, to 35% in those ≥ 35 years. Rates of hepatitis A and B antibodies among 70 military recruits (mean age 20 years) were similar to those among regular forces. In this young healthy population, exposure to hepatitis A before the age of 18 is almost universal, while exposure to hepatitis B is related to age and ethnic origin.

HEPATITIS B VACCINE TRIALS IN THE BDF

The study entitled, "Comparative Trial of Low-dose, Intramuscular, Recombinant Hepatitis B Vaccine in the Belize Defense Forces", involves three injections and three blood draws of BDF soldiers over an eight-month period. Commander Joe P. Bryan, MC, USNR, immunized 301 soldiers (negative for anti-HBc) in September 1992 and 290 of these in October 1991.

During late November and early December 1992, ERC personnel assisted Dr. Craig with drawing the first blood sera (two months

post initial injection). A third injection is scheduled for March 1993, concurrent with a second blood draw, to be followed in May 1993 with the third and final blood draw.

Mosquito (Malaria Vector) Ecology

Anopheles darlingi, a major vector of malaria in large regions of Central (including Belize in previous surveys) and South America, and *An. vestitipennis* were not found in Belize during the initial larval surveys in the Corozal, Orange Walk, Belize City, Cayo and Stann Creek districts¹⁸. In a recent wet season survey in Toledo district, nighttime, paired indoor-outdoor landing collections from humans were included to increase the likelihood of detecting the presence of *An. darlingi* and *An. vestitipennis* mosquitoes. No specimens of *An. darlingi* were encountered during this survey. The dominant species indoors was *An. vestitipennis* and the second most common species was *An. albimanus*. Both *An. albimanus* and *An. vestitipennis* were widely distributed, being present at 6 of 6 and 5 of 6 collection sites, respectively. More specimens of *An. albimanus* and *An. apicimacula* were collected outdoors than indoors. In contrast, 86% of all *An. vestitipennis* females were collected indoors. These findings are indicative of possible changes in vector roles in Belize and need to be taken into consideration when planning mosquito spraying strategies.

Anopheles albimanus is the most common of four anopheline species present in the study area. It occurs mostly on the coastal plain and is present in both the wet and dry season. *An. albimanus* may share a habitat with *An. crucians*. *An. pseudopunctipennis* and *An. argyritarsis* were present during the dry season and their distribution seems to be limited to the Karst and Mountain Pine Ridge regions.

During the dry season, *An. albimanus* was positively correlated with blue-green algae mats and submersed/periphyton habitat types, and negatively correlated with filamentous algae habitat type. *An. crucians* was positively correlated with *Eleocharis*/periphyton habitat type. *An. pseudopunctipennis* and *An. argyritarsis* were positively correlated with filamentous algae habitat type, and *An. argyritarsis* was also positively correlated with the rock pool habitat type^{18,19}. Discriminant functions based on environmental variables showed very good prediction for *An. pseudopunctipennis* and *An. crucians* populations.

Tick borne fever

The results of the examination of single sera for IgG antibodies to various antigens accomplished at USAMRIID, from workers and students at Caracol are shown in Table 6.

Table 6. Prevalence of viral and bacterial IgG antibodies in workers and students at Caracol.

<u>Antibody</u>	<u>No. Positive</u>
EEE	8
VEE	13
DEN2	16
SLE	12
CHG	0
P360	1
HTN	1
FTS	3
LEPTO	9
HIV	0
SYPHILIS	3
LYME	7

The preliminary results showed 7 individuals positive for Lyme disease. Three of these specimens are also positive for syphilis, a known cross reactor. There are a number of antibodies to other infectious diseases which will cross react in this enzyme linked immunosorbent assay; therefore, the results need to be confirmed with other diagnostic tests; i.e., indirect fluorescent antibody and polymerase chain reaction.

CONCLUSIONS

Too few patients have been studied to date to draw inferences about major causes of FUO and jaundice; however, the prevalence of antibodies to a wide range of infectious disease agents of interest is high. Seroprevalence surveys are necessary to determine the geographic regions and populations where additional surveillance and prevention efforts are warranted.

Leishmaniasis is endemic throughout the forested areas of Belize. The incidence of leishmaniasis in BDF and BFB troops should continue to be monitored, and when necessary, interventions should be introduced. A comprehensive study of leishmaniasis should be started to define the epidemiology of the disease more completely in Belize.

Gastroenteritis in Belize apparently has a seasonal pattern dissimilar to other countries in the region. The incidence of cases will be closely monitored throughout the year.

The incidence of hepatitis in the villages studied in the Stann Creek District is high. The behaviors and risk factors that leave this population to be particularly prone to hepatitis infection are a matter of speculation. We have knowledge of problems with water and sanitation, but we know little about the health and cultural practices, beliefs and attitudes of these communities that relate to the blood-borne spread of hepatitis. Other variables must be known to plan an effective intervention strategy that will enhance the long-term health status of this population. We have an indication that parenteral antimalarials, vitamins, antibiotics and fluids are used in the absence of physician supervision and that dental practices occur outside a professional setting. Thoroughly documenting the threat for blood-borne disease transmission deserves a high priority.

Surveys of mosquito larval habitats have been conducted in the northern part of Belize in both the wet and dry seasons. Although we are seeing great ecological variability from one area to another, we are also beginning to see consistency in vector preferences for habitats and habitat-types. As more standardized data is compiled within the geographic information systems, augmented with remote sensing data, habitat data will be developed that, hopefully, will be predictive of where and when these vector populations will occur. A third faunistic survey will be conducted during the upcoming dry season to verify and enhance previously collected data.

The cause of illnesses in the archaeological team has not been definitively identified.

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O-8

POLYMERIC DRUG DELIVERY SYSTEMS: CHANGING THE FACE OF COMBAT CASUALTY CARE

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Polymeric drug delivery systems offer enormous potential for improved trauma management in both the military and civilian sectors. At USAIDR there is intense interest in developing innovative polymer technologies to improve methods of care for craniofacial combat casualties. Advances are being made in two critical areas—wound infection control and bone repair. In preclinical trials, polymer-encapsulated antibiotics applied locally have proven superior to conventional systemic therapy for wound infections in soft tissue and bone. The most mature prototype, microencapsulated ampicillin, is now in advanced development. Research continues on encapsulation of follow-on antibiotics, such as aminoglycosides and cephalosporins. These encapsulates, when combined in a broad-spectrum antimicrobial "cocktail", will control all wound-contaminating microorganisms with a single, sustained-release dose. Along with sustained infection control, restoration of form and function following combat-incurred craniofacial trauma often requires replacement of lost bone. Currently available grafts and implants present deficiencies which are avoidable if bone can be regenerated, rather than replaced, in the defect site. Researchers at USAIDR are pursuing development of biodegradable polymeric matrices which can function as scaffolds for guided bone regrowth, as vehicles for sequential delivery of bone-inductive proteins and, ultimately, as carriers for cultured bone-precursor cells. Design of these sophisticated systems demands creative polymer synthesis, engineering, and analytical strategies. The goal is to provide military surgeons with effective off-the-shelf treatments that can be deployed far forward in the combat theatre. These new drug delivery systems will contribute to improved patient care beyond the craniofacial complex and beyond the battlefield.

O-10

LEAD-BASED PAINT HEALTH RISK ASSESSMENT

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Lead toxicity is a man-made disease, a product of industrialization due to the element's use in paint, batteries, insulation and gasoline. Lead remains as a nonbiodegradable environmental contaminant unless carefully removed. In children, lead causes serious permanent damage as a neurotoxin. During scheduled Annual Training, the 363d Medical Laboratory Command responded to the Fort Devens commander's request for the design and implementation of a lead-based paint health risk assessment to be conducted with the DEH and MEDDAC both in installation housing and the ongoing blood screening for lead in children age six and under.

Modified Housing and Urban Development (HUD) guidelines were employed in the assessment of all 1,723 housing units on post. Factors used included, but were not limited to, age and condition of the structure and age, health, hygiene and blood lead levels of the occupants. All soldiers received comprehensive training from state licensed lead abatement experts and experts extant within the laboratories. Though incomplete, preliminary data support the conclusion that the potential for exposure is relatively low. These data indicate 68.6% of the housing units with a low risk of exposure, 31% had a medium risk, and 0.4% had a high risk. Blood lead levels were within current standards.

Health and safety standards concerning threshold limit values (TLV) of lead remain in a state of flux; governmental requirements are either inconsistent or under reevaluation. Until data clearly support a TLV no environmental or blood

O-9

GASTROENTERITIS OUTBREAK IN BELIZE

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An outbreak of gastroenteritis was identified in the northern districts and cayes of Belize in February 1991. Forty five of the 467 cases reported through mid March were tested for the presence of rotavirus antigen. Of these, 23 (51%) were positive. The reported seasonal increase in gastroenteritis in the region occurs during October and November; however, an analysis of 1605 cases of gastroenteritis reported since October 1988 at a clinic in Belize City, indicated that the outbreak was seasonal, with an increase in incidence January - May and a peak incidence January - March. The seasonality coincides with the rainfall pattern in Belize, supporting the supposition that the mode of transmission for rotavirus is fecal-oral. Over 69% of the reported cases in the outbreak and approximately 45% of the historical cases were in infants and children ≤ 4 years of age. The percentage of cases in individuals >4 years of age is higher than would be expected in an outbreak due solely to rotavirus, suggesting additional etiologies.

O-11

ROLE OF PROTECTED BRUSH CATHETERS IN THE DIAGNOSIS AND MANAGEMENT OF ADULT PATIENTS WITH BRONCHIECTASIS

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Patients with bronchiectasis may present with subtle features and empiric antibiotic therapy may be problematic. We have evaluated 11 patients with a bronchoscopic protected specimen brush (BPSB) technique in the setting of bronchiectasis. All patients had compatible clinical features, and in 10 the diagnosis was confirmed roentgenographically. One patient presented particularly interesting microbiologic results. The patient is a 78 year old man, who had carried diagnostic bronchiectasis since 1985. Multiple sputum cultures were negative. He however showed persistent clinical deterioration. Upon hospital admission sputum gram's stain showed greater than 100 white blood cells (WBC) per low power field (LPF), moderate gram positive cocci and moderate gram negative rods. Sputum culture grew *Viridans* group streptococci and gamma hemolytic streptococci. Quantitative culture grew 6×10^6 cfu/ml *Acinetobacter baumannii* (formerly *A. calcoaceticus* subsp. *anitrans*); 9×10^3 *Pseudomonas aeruginosa* and 3×10^5 *Pseudomonas fluorescens*. Therapy directed toward these isolates resulted in significant clinical improvement.

Initial report of a hepatitis investigation in rural Belize

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Abstract

In spring 1991, Belizean health officials expressed concern about a possible hepatitis outbreak in a banana farming district. A study was designed to identify cases and to address the serological prevalence of hepatitis virus markers. Three populations were studied: (i) persons meeting a clinical case definition for hepatitis; (ii) designated banana workers; and (iii) people in a random sample of households in the community. Information was collected using questionnaires and sera were collected for laboratory testing. This report presents the preliminary results of a study conducted in June 1991. Among people who met the clinical case definition, 24% of 42 tested had immunoglobulin M antibody to hepatitis B virus (HBV) core antigen (anti-HBc IgM). In the worker and household survey populations, 284 and 280 people, respectively, were tested for anti-HBc IgM. In each group, 4% were positive. HBV surface antigen was found in 37% of 43 clinical cases, 18% of workers, and 13% of people in the household survey. Among the 3 study populations, the prevalence of HBV core antibody (anti-HBc) ranged from 73% to 81%. Almost all tested persons had evidence of prior hepatitis A virus infection. Evidence of prior infection with hepatitis viruses A and B was widespread, but an aetiology could not be established for most of the clinical cases. However, the prevalence of hepatitis B markers in this population was very high compared to other reports from the Caribbean.

Introduction

In May 1991, concern that cholera might be spreading north through Central America prompted officials of the Belizean Ministry of Health to initiate a cholera prevention and control campaign. In the Cowpen area of south-east Belize, there was no report of illness resembling cholera; however, banana farm workers and local health officials expressed concern about allegations that large numbers of people were or had been ill with hepatitis. Some people had diagnosed themselves as having hepatitis, based upon the occurrence of jaundice. In others, the diagnosis had been made by local health professionals.

Cowpen residents considered the appearance of hepatitis to be an annual occurrence associated with the dry season; they attributed the disease to poor water quality. However, many believed that the 1991 dry season had been more severe and prolonged than usual and that the number of hepatitis cases, particularly in young adults, was greater than that observed in past years. Concern heightened when a young pregnant woman allegedly died with hepatitis.

When the Belizean Ministry of Health requested assistance in investigating the alleged outbreak, we conducted a preliminary investigation in May 1991 to establish the cause(s) of the reported illnesses. The May investigation confirmed the presence of hepatitis in the Cowpen area, based upon abnormal liver function tests and serological studies for hepatitis virus markers. This was followed in June 1991 by an expanded effort to identify cases and to make a cross-sectional study for hepatitis markers in banana farm workers and their families. This is the initial report of the June 1991 investigation. Serological studies and data analyses are continuing, and results of work still in progress will be reported later.

Materials and Methods

Three study populations were defined: cases, banana workers, and households. Individual, household and farm questionnaires were developed in English and Spanish for the uniform collection of information. All individuals were given unique identifiers, which were assigned to serum collection tubes and questionnaires.

A case of hepatitis was defined as anyone who, in the preceding 6 months, based upon the review of a study team physician, had been diagnosed as having hepatitis

by a health care worker or had experienced jaundice. Possible cases were identified for review by a team physician through (i) local health worker reports of currently or previously ill people, (ii) reports from farm and civic leaders of ill people, (iii) study team physicians holding sick call and making house calls on request, and (iv) study team members asking every adult contacted if they had been jaundiced or diagnosed as having hepatitis, or knew of anyone who had. After a study team physician determined that a person met the case definition, serum was collected and a case questionnaire was used to obtain illness data not covered in other questionnaires.

During May-June 1991, local officials in Cowpen estimated the total population of banana farm managers, banana field and shed workers, and family members to be 1300 to 1500 people. These people worked on 7 different banana farms and lived in 8 worker housing areas, all of which were identified for study. Farm personnel usually were studied at the farm packing shed and were questioned about demographic variables, the occurrence of illness, and variables associated with hepatitis virus transmission. An attempt was made to study all banana farm workers. However, 2 factors limited the percentage studied: (i) when banana harvesting and packing were occurring at high intensity, many workers could not be released to participate in the study; and (ii) when there was a lull in harvesting and packing, many workers immediately left the area to visit friends and relatives in Belize or in their homeland. At each household, a household census with demographic information was obtained, and questions were asked about the occurrence of illness and variables associated with hepatitis virus transmission.

Blood for serological studies was obtained from all consenting individuals over 4 years of age. Four years was established as the cut-off age because of reluctance on the part of most parents to have blood drawn from younger children. Response refusals were rare.

An attempt was made to test all sera for antibody to hepatitis A virus (anti-HAV), immunoglobulin M (IgM) antibody to hepatitis A virus (anti-HAV IgM), hepatitis B virus (HBV) surface antigen (HBsAg), antibody to HBV core antigen (anti-HBc), IgM antibody to HBV core antigen (anti-HBc IgM), and antibody to hepatitis C virus (anti-HCV). Only sera positive for HBsAg were tested for HBV e antigen (HBeAg) and antibody to hepatitis delta virus (anti-HDV). All clinical cases were

from cases in which there was no serological evidence of infection with any of the hepatitis viruses also were tested by enzyme-linked immunosorbent assay (ELISA) for IgG and IgM antibodies against leptospira and 2 strains of hantavirus.

Anti-HAV, anti-HAV IgM, and anti-HDV were determined using ELISA kits from Abbott Laboratories (North Chicago, Illinois, USA). Testing associated with HBV also was done using Abbott ELISA kits. Ortho Diagnostics (Raritan, New Jersey, USA) ELISA kits were employed to determine the presence of antibody to HCV. Both the first generation anti-HCV tests and the second generation immunoblot assay for the detection of antibody to HCV (RIBA-HCV) were utilized. Antibody to HEV was detected using a prototype ELISA GOLD SMITH *et al.*, 1992; SKIDMORE *et al.*, 1992).

Data were entered into an EpiInfo database (DEAN *et al.*, 1990). Check files and double entry were used to ensure valid transfer of hard copy questionnaires to the data files. EpiInfo was also used to develop descriptive statistics.

Results

A summary of the serological testing results for hepatitis markers is presented in the Table. The clinical case, worker and household survey populations were not mutually exclusive.

77%) had anti-HBc, indicating prior HBV infection.

Of 482 inhabited dwellings in the 8 housing areas, 146 (30%) were randomly selected and studied. The household sample subgroup included 285 people. The mean age was 26 years (SD=15); 152 (53%) were males. Almost all (275, 98%) had anti-HAV. The 2 children with recent HAV infection described in the clinical case group are also shown in the household sample in the Table. Ten (4%) had anti-HBc IgM, and 37 (13%) had HBsAg. Twenty (54%) people with HBsAg also had HBeAg. As in the other subgroups, most (203, 73%) had evidence of past HBV infection.

In all population subgroups studied, only very small numbers of people were found to have had contact with HCV or HEV. There was no evidence of exposure to HDV or recent infection with leptospira or hantavirus.

Discussion

We found a high prevalence of people with evidence of prior infection with HAV and HBV, a high prevalence of HBsAg positivity, and evidence for recent HBV infection in 24% of the people with clinical illness. Overall, evidence of HBV infection was found in 73% of the people studied, and 16% were positive for HBsAg. The prevalence of anti-HCV was low, and no evidence was found for HDV infection.

Nearly all people studied had anti-HAV. In contrast,

Table. Summary of results of serological testing for hepatitis markers, Cowpen, Belize, 1991

Specific marker ^a	Clinical cases n=47		Banana workers n=290		Household sample n=285	
	No. tested	No. positive	No. tested	No. positive	No. tested	No. positive
Anti-HAV	43	41 (95%)	284	277 (97%)	280	275 (98%)
Anti-HAV IgM	43	2 (5%)	282	0	278	2 (1%)
HBsAg	43	16 (37%)	284	50 (18%)	280	37 (13%)
HBeAg ^b	15	4 (27%)	49	13 (26%)	37	20 (54%)
Anti-HBc	42	34 (81%)	284	219 (77%)	278	203 (73%)
Anti-HBc IgM	42	10 (24%)	284	12 (4%)	280	10 (4%)
Anti-HCV	43	1 (2%)	284	3 (1%)	280	3 (1%)
Anti-HDV ^b	14	0	48	0	32	0
Anti-HEV ^c	38	2 (5%)	24	2 (8%)	18	0

^aHAV=hepatitis A virus, IgM=immunoglobulin M, HBsAg=hepatitis B virus surface antigen, HBeAg=hepatitis B virus e antigen, HBc=hepatitis B virus core antigen, HCV=hepatitis C virus, HDV=hepatitis D virus, HEV=hepatitis E virus. Insufficient quantities of sera prevented complete testing of all people studied.

^bOnly people positive for HBsAg were tested. Expressed percentages are based upon the number of HBsAg positive people tested.

^cOnly clinical cases were tested. Some cases were workers; some were also part of the random household sample.

Forty-seven people met the clinical case definition of hepatitis. Their mean age was 26 years (SD=14); 29 (62%) were males. Twenty-nine cases were identified as workers, and 25 (86%) were male. The average age of worker cases was 32 years (SD=11).

Laboratory results were obtained for 43 cases. Two children were too young for drawing blood and 2 serum specimens were lost. Forty-one (95%) of the cases were positive for anti-HAV, and 2, both children, had anti-HAV IgM, indicating recent HAV infection. Ten (24%) were newly infected with HBV, as demonstrated by the presence of anti-HBc IgM, and 16 (37%) were HBsAg positive. Among those with HBsAg, 4 (27%) also had HBeAg. Evidence of past HBV infection (presence of anti-HBc) was found in 34 (81%).

A total of 512 male and 111 female banana workers was identified. Of these 623, 290 (46%) were studied and blood was tested from 284. In the subgroup of 290 workers, the mean age was 32 years (SD=13); 228 (79%) were males. There was no indication of recent HAV infection, but 277 (97%) had anti-HAV, indicating prior infection. Twelve workers (4%) had anti-HBc IgM and 50 (18%) had HBsAg. Among those with HBsAg, 13 (26%) also had HBeAg. The majority of workers (210

anti-HEV could be demonstrated in only 2 cases and it was not possible to distinguish between recent and past infection. The study of sera from Cowpen for evidence of HEV infection is continuing because ELISA tests for anti-HEV are still in the early stages of development (GOLD SMITH *et al.*, 1992; SKIDMORE *et al.*, 1992); sensitivity and specificity have not been completely determined. Work is under way to identify any antigenic variation among hepatitis E viruses. Because we could not establish a serological diagnosis for most of our hepatitis cases, these might have been due to HEV or another agent of non-A, non-B hepatitis.

In the absence of a demonstrable viral agent, consideration must be given to toxic substances, including ethanol or a contaminated food supply. Alcohol consumption was noted but collection of these data was outside the scope of the present study. There was no indication of disruption in the food supply during the dry season to suggest that hungry people might have consumed grains or other foods heavily contaminated with fungi and fungal toxins. Epidemiological analysis of a vast amount of demographic data collected for people studied is in progress, with a wide range of possible aetiologies being considered.

HAV and HEV have faecal-oral routes of transmission and problems existed with both drinking water quality and sanitation in Cowpen. Our study indicated that hepatitis A in Cowpen is an illness of children. HEV may have been recently introduced, thus explaining at least some of the cases of non-A, non-B hepatitis in young adults. Prevention of both HAV and HEV infections is strongly dependent upon a purified water source, good personal hygiene, and proper disposal of human waste. After attention was directed toward the probability of enterically transmitted hepatitis virus or viruses in Cowpen, some farm managers and local inhabitants built improved water systems that included treatment measures.

In screening refugees to the USA from Asia, Africa and eastern Europe, investigators have reported HBsAg prevalences ranging from 1.1% to 15.5% (CRYSEL *et al.*, 1991). Along the southern border of Mexico, another study reported the seroprevalence of HBsAg to range from 4.2% to 17.3%. This prevalence is uniquely high among the reported prevalences from the Caribbean nations. Overall, 91% of that Mexican population had been infected with HBV, and HDV was present in 50% of those who were HBsAg positive (ALVAREZ-MUNOZ *et al.*, 1989).

Within Central America, the lowest reported prevalence of HBsAg (0.64%) was found in Costa Rica (SALOM *et al.*, 1990). Throughout the Americas, from 1975 to 1985, HBsAg prevalence was reported to range from 0.3% in the USA to 8.0% in Amazonia. The countries surrounding Belize have a reported HBsAg prevalence between 1% and 3% (FAY *et al.*, 1990). However, the true impact and prevalence of viral hepatitis throughout many countries in Central and south America have yet to be studied (PAHO, 1990; WHO TECHNICAL ADVISORY GROUP ON VIRAL HEPATITIS, 1988). Cowpen HBV marker prevalences match or exceed the highest 1980 prevalences reported in Senegal, Thailand, Uganda, Egypt, and India (SOBESLAVSKY, 1980).

In the Cowpen population, with a very high prevalence of HBV markers, we found little evidence of HCV infection. HBV and HCV are transmitted through direct contact with blood, blood products and possibly other body fluids. However, infection with HCV appears to occur independently from HBV and is a frequent complication of transfusions (HAYASHI *et al.*, 1991). HBV can be acquired as a result of intravenous drug use with shared needles, blood transfusion and sexual contact. Individuals infected with HBV who are positive for HBeAg are considered highly infectious. HBeAg was found in 26%, 27%, and 54% of those HBsAg positive within the three populations we studied.

The specific risk factors that made the Cowpen population particularly prone to HBV infection are still under study. We do have information that parenteral antimalarials, vitamins, antibiotics and intravenous fluids were used without a physician's supervision, and that dental practices occurred outside a professional setting. Overall, people had a high level of interest in their health and the health of their family and friends. Generally recognized measures for preventing HBV infection include passive/active immunizations and counselling for behavioural modification. Efforts were initiated in 1991 in Belize to enhance the screening of blood donations for hepatitis markers. Screening of pregnant women for HBV infection must also be emphasized.

The high prevalence of HBV markers in the Cowpen population is also cause for concern about the potential for transmission of other blood-borne pathogens such as human immunodeficiency virus (HIV) and other retroviruses. Although human T cell lymphotropic viruses 1 and 2 have been found in 9% of one Indian tribe in Panama, it is not clear how transmission occurred (REEVES *et al.*, 1990). HIV infection is devastating the population in many of the same countries that reported high HBV rates 10 years ago. Greater knowledge about transmission characteristics is critical to the development of preventive

measures against such agents.

Our study emphasizes the need to learn more about the transmission of hepatitis viruses in rural Belize in order to institute specific intervention efforts. It is essential to determine when and where infection occurred since the banana workers of Cowpen were not a stable population. Most people studied came from surrounding countries and lived in Belize for variable periods. Additionally, our cross-sectional study was done when there may have been an increase of hepatitis in the region. We may have looked at a rate of infection which reflected exposure in Belize, outside Belize, or both.

In summary, we found a high prevalence of HBV infection markers and an unusually high prevalence of HBsAg positivity in Cowpen, Belize. Additionally, almost all people had evidence of HAV infection by the time adulthood was reached. Both hepatitis A and B are preventable through well established environmental controls and personal behavioural changes. Detailed descriptive epidemiological studies followed by intervention initiatives are needed to reduce hepatitis virus transmission and the associated morbidity.

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TITLE PAGE

Running Title
Prevalence of Hepatitis A and B in Belize

Full Title
THE PREVALENCE OF HEPATITIS A AND B INFECTION AMONG
DIFFERENT ETHNIC GROUPS IN BELIZE

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to be considered official or to necessarily represent the views of the
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Abstract

Little is known about the prevalence of infection with hepatitis viruses in Belize, Central America. We conducted a serologic survey among members of the Belize Defence Force (BDF), which is comprised of the five major ethnic groups in Belize, in order to estimate prevalence rates of hepatitis A and B among military aged men and women in Belize. Of approximately 600 men and women in the BDF, 394 (66%) volunteered to participate. Antibody to hepatitis A was found in 95%, with similar rates by age, rank and ethnicity. Antibody to hepatitis B core antigen (anti-HBc) was found in 31%. Rates of anti-HBc varied significantly among the ethnic groups with the lowest rates in Mayan Indians (5%) and Mestizo (7%), with significantly higher rates among Creole (30%) and Garifuna (54%). Rates increased with increasing age from 25% in those 18-24 years to 35% in those ≥ 35 years ($P = .06$, chi-square for trend). Hepatitis B surface antigen was found in 15 (4%) overall. Rates of hepatitis A and B antibodies among 70 military recruits (mean age 20 years) were similar to those among regular forces; anti-HAV was found in 91%, anti-HBc in 34%, and HBsAg in 6%. In this young healthy population, exposure to hepatitis A before the age of 18 is almost universal while exposure to hepatitis B is related to age and ethnic origin.

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Environmental and regional determinants of anopheline larval distribution in northern Belize

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Abstract

Surveys of anopheline breeding sites were conducted in northern Belize during September 1990 and April 1991. Detailed phytoecological descriptions were recorded for each collection site. Larvae were reared to adults and larval and pupal skins were preserved as taxonomic series. Five anopheline species were collected and data on the presence or absence of four species were adequate for analyses of phytoecological associations. Results for *Anopheles albimanus*, *A. crucians*, *A. pseudopunctipennis* and *A. argyritarsis* larvae are included in this report. Environmental variables significantly associated with the presence of these anophelines were selected for use in discriminant functions (DF). The accuracy of the DFs were evaluated by crossvalidation. Variables used for *A. pseudopunctipennis* were filamentous algae, altitude and water depth. The DF was 100% and 93% accurate in predicting positive and negative habitats, respectively. Variables in DF for *A. albimanus* were submersed macrophytes, cyanobacterial mats, altitude and temperature. In crossvalidation tests, 45-89% and 81-95% of positive and negative habitats were accurately predicted, respectively. Periphyton, detritus, emergent macrophytes and habitat area were variables used in DFs for *A. crucians*. The accuracy of predictions was similar to the results with *A. albimanus*, which reflects the wide tolerance of different habitats by both species. No DF was constructed for *A. argyritarsis* since it was found only at high altitudes in rock pools and pools with filamentous algae. In a more holistic approach to this study we described habitats according to dominant vegetation, classified them into habitat-types and examined the association between habitat-types and presence or absence of larvae. Additionally, the associations of habitat-types with regions was analyzed. Differences between seasons and the biological implications of these findings are discussed.

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**Preliminary observations on the changing roles of malaria
vectors in southern Belize¹**

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The presence of *Anopheles darlingi* Root in Belize was first reported by Komp in 1940. In follow-up studies during 1940, Kumm and Ram (1940) documented the occurrence of house-frequenting populations of *An. darlingi* in the Toledo and Stann Creek districts of Belize (see map). Kumm and Ram relied heavily on searches of houses for resting mosquitoes as their primary survey method. In Stann Creek district, *An. darlingi* was found in 5 of 7 localities surveyed and in 3 of 7 localities in Toledo district. These investigators also collected larvae of *An. darlingi* in both districts. They found two species with malaria sporozoites in their salivary glands, viz, *An. darlingi* and *An. vestitipennis*. *Anopheles darlingi* was the dominant indoor anopheline in the Stann Creek and Toledo districts, representing over 70 % of the anophelines caught indoors in rural areas. *Anopheles albimanus* was the most widely distributed species, being present at 9 of 14 localities from both districts. *Anopheles vestitipennis* was found at 3 of 7 localities in Stann Creek district, but was not found in Toledo district.

The Kumm and Ram survey was conducted over 50 years ago, prior to the use of DDT in the malaria control program in Belize. Since their survey, no comparable data have been published for the Toledo and Stann Creek districts.

We initiated a malaria vector research program in Belize in 1990 and conducted extensive larval surveys in the Corozal, Orange Walk, Belize City, Cayo and Stann Creek districts. No *An. darlingi* or *An. vestitipennis* mosquitoes were collected in those surveys (Rejmankova et al. 1993). In a recent wet season survey in Toledo district we included nighttime, paired indoor-outdoor landing collections from humans to increase the likelihood of detecting the presence of *An. darlingi* and *An. vestitipennis* mosquitoes. These collections were performed by collecting mosquitoes as they landed on the exposed legs and feet of collectors. Paired indoor-outdoor collections were conducted one night at each of six localities from 6:30 to 7:15 p.m. Based on previous experience (Roberts et al 1987), the sunset interval should be a period of peak *An. darlingi* host-seeking activity. Although we employed uniform collecting methods, we could not control for numbers of children and adults who gathered around the collectors at both indoor and outdoor collecting sites.

Collections were performed at 6 sites in the area of Punta Gorda (Toledo district) in southern Belize (see map). Although collections were conducted in the same general areas surveyed by Kumm and Ram (1940), their specific sites were no longer in existence. In addition to the nighttime landing collections, we conducted larval collections at 145 sites in Toledo district.

No specimens of *An. darlingi* were encountered during this survey. The dominant species indoors was *An. vestitipennis* and the second most common species was *An. albimanus* (Table 1). Both *An. albimanus* and *An. vestitipennis* were widely distributed, being present at 6 and 5 sites, respectively. More specimens of *An. albimanus* and *An. apicimacula* were collected outdoors than indoors. In contrast, 86 % of all *An. vestitipennis* females were collected indoors. All three *Anopheles* collected in landing captures were also represented in larval collections.

Table 1. Numbers of anophelines collected from humans in paired indoor/outdoor landing collections in the Punta Gorda area of southern Belize during August and September, 1992.

Collecting Site	<i>An. albimanus</i>		<i>An. vestitipennis</i>		<i>An. apicimacula</i>	
	inside	outside	inside	outside	inside	outside
Jacinto Landing	2	3	27	4	0	0
Santa Helena	2	1	0	0	0	2
Crique Mafredi*	5	2	39	7	7	29
Crique Trosa*	0	2	9	0	9	33
Punta Gorda	0	2	2	1	0	0
Big Fall	1	25	0	1	0	0
Totals	10	35	77	13	16	64

Collection data presented herein indicates that *An. darlingi* is very uncommon or possibly absent in the Punta Gorda district. In either case, it is not likely to be an important factor in the continued transmission of malaria in Punta Gorda. While *An. vestitipennis* was not encountered in the district during 1940, it is now the numerically dominant *Anopheles* inside of houses. An intriguing aspect of this species' behavior relates to our capturing many more inside of houses than were captured outdoors. While these observations are preliminary, the f.a. knows of no other New World *Anopheles* which demonstrates a comparable level of endophagy. Collections of large numbers of *An. vestitipennis* inside of both DDT-sprayed and unsprayed houses is another interesting observation. In contrast, the host-seeking females of *An. apicimacula*, like *An. albimanus*, were much more abundant outdoors. Exophagic behavior of the latter two species should serve to diminish their overall vectorial capacity.

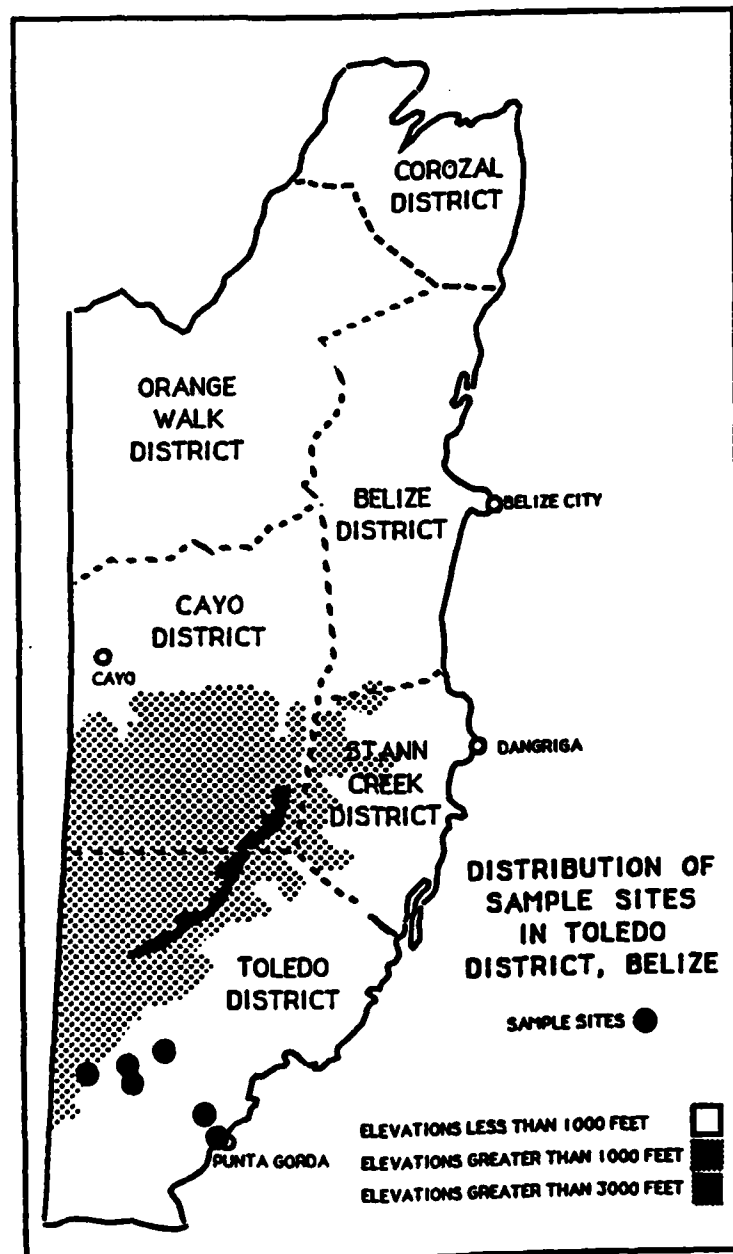
The presence and abundance of malaria vectors are under the control of dynamic environmental variables, as well as human interventions. This report emphasizes the need to continuously study the changing roles of malaria vectors in different geographical areas. Based on the published literature, we can expect *An. darlingi*-transmitted malaria to respond favorably to a DDT house-spray program (Rozendaal et al 1989, Roberts and Alecrim 1991). However, these expectations must be reevaluated if *An. darlingi* has been replaced (as the vector of malaria) by *An. vestitipennis*. Hopefully this report will stimulate additional studies on vectorial roles and vector responses to the DDT-house spray program in different ecological zones of Belize.

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* Houses not sprayed with DDT.

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Map of Belize depicting the distribution of collecting sites in Toledo district, near Punta Gorda, Belize.